# PERMEABILITY OF THE TRACHEAL SYSTEM OF *DROSOPHILA MELANOGASTER* LARVAE[1]

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It has in the past been commonly assumed that the absorption of fluids and gases through the insect tracheal system occurred through the finest branches of the system, the tracheoles (Wigglesworth, 1933), or through the large tracheal end cells (Lund, 1911). In 1938, Wigglesworth found that in one mosquito larva, of the hundred experimented upon, the entire tracheal system was permeable to indigo carmine. From this it was concluded that the tracheal system of the mosquito larva is permeable to gases. It has been shown that fluid is normally present in the tracheoles of several insects (fleas, mosquito larvae), that the tracheolar fluid plays some part in the absorption of oxygen from the tracheoles by the tissues, and that the level of liquid varies with the activity of the insect. During moulting the walls of the tracheal system are more readily permeable to fluids and gases than they are during the intermoulting period (Wigglesworth, 1939). Data here presented show that the entire tracheal system of the larva of *Drosophila melanogaster* is at all times permeable to fluids and gases.

## TECHNIQUE

The larvae used were those of the wild type *Drosophila melanogaster,* usually 2nd and 3rd instar, approximately 5 to 15 mms in length. The larvae were raised in milk bottles on a mixture of corn meal, molasses, and agar (Bridges, 1932), in an incubator at a temperature of 25 degrees C. Before being used, the larvae were withdrawn from the bottle in a medicine dropper full of saline solution, washed in a beaker of saline, and dried with filter paper. When dry each larva was placed on a microscope slide and held in place with a piece of cellulose "scotch" tape. The advantages of using the "scotch" tape are several: (1) it is permeable to gases and permits the animal to breath freely, (2) it permits the application of gases and fluids and other stimuli during experimentation, (3) it has the same refractive index as glass, making observations of the tracheal system easily possible[2], and (4) it

[1]This work was accomplished at Tuft's College, under the supervision of Prof. K. D. Roeder.

[2]The tracheal system, when filled with air, appears silvery by transmitted light as compared with the other tissues which are almost transparent; upon entrance of fluid into the system the tracheae become almost transparent.

prevents the larva from wriggling out of the field of the microscope.

Various agents were tried and their effect upon the appearance of fluid in the tracheal system observed. Observations were made under all powers of the microscope.

## Results

A summary of the results obtained is given in Table 1. In the normal *Drosophila* larva, no liquid could be found in the tracheal system, not even in the finest branches that could be seen with the microscope. Tactile stimulation, which consisted of either rolling the insect over two or three times before placing it on the slide, or gentle stroking with fine forceps, resulted in the appearance of fluid more than did any other stimulus. The fluid entered the tracheal system at various points. In some cases the entry occurred only in the tracheoles, but at other times it entered the main tracheal trunks first, and sometimes it entered several branches at once. The entry of fluid was sometimes, but not always, accompanied by collapse of the tracheae. Electrical stimulation caused fluid to appear in only one out of ten larvae used. The fluid entered in all branches except the commissures. Passing ether or carbon dioxide through the "scotch" tape caused liquid to enter the tracheal system quite easily. In several cases, leaving the larvae in a dish of saline solution for several days also resulted in the appearance of fluid in the tracheae.

TABLE 1

The Effect of Various Agents Upon the Appearance of Fluid in the Tracheal System of *Drosophila melanogaster* Larvae

| Stimulus | No. of Larvae Used | No. of Larvae Responding | Points of Entry of Fluid | | | | |
|---|---|---|---|---|---|---|---|
| | | | Main Trunks | Segmental Branches | Inter-segmental Commissures | Trach-eoles | Dorsal Commis-sures |
| Tactile | 15 | 10 | 3 | 4 | 4 | 7 | 1 |
| Saline | 10 | 3 | 2 | 2 | 0 | 0 | 1 |
| Ether | 16 | 8 | 4 | 6 | 2 | 2 | 1 |
| Carbon dioxide | 10 | 5 | 2 | 3 | 1 | 2 | 1 |
| Electrical | 10 | 1 | 1 | 1 | 0 | 1 | 0 |
| Death | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Normal | 10 | 0 | 0 | 0 | 0 | 0 | 0 |

Neither the heart rate nor the spiracular rate changed from normal during the time fluid was in the tracheal system. Time of reentry of fluid from the tracheal system into the tissues varied from a few minutes to twenty four hours. In one case a drop of liquid was observed within the tracheolar lumen at the junction of three tracheoles of about 0.3 micra diameter. The fluid was seen to enter the tissues in fifteen minutes, accompanied by pumping movements of the three tracheoles involved. This was the only case in which pulsation of the tracheoles was observed. Nor was there any pulsation of the main trunks during normal respiration (as described in the flea by Herford, 1938). After death the entire tracheal system filled rapidly with fluid with no specific point of entry.

The implications of the results obtained are several: (a) the entire tracheal system of the larva of *Drosophila melanogaster* is permeable to fluids and gases, and since according to Krogh (1941), ''a high degree of permeability for oxygen, is, as far as we know, not physically possible without permeability for water'', respiratory gaseous exchange can and probably does occur throughout the entire system, rather than exclusively through the tracheoles or tracheal end cells, (b) there is apparently no difference in the structure and chemical composition of the tracheae and tracheoles.[3]

There is no definite answer to the questions of the significance of fluid in the tracheal system or what mechanism causes the fluid to appear. It has been discovered that the fluid in the tracheoles of mosquito larvae moves back and forth in relation to the amount of oxygen needed by the tissues. When the insect is at rest the tracheoles contain a large amount of fluid, but this, when the insect is active, is absorbed by the tissues and air can replace it in the tracheoles and reach the tissues by diffusion (Sykes and Wigglesworth, 1931). The withdrawal of fluid was attributed to an increase in osmotic pressure in the tissues with consequent flow of fluid from the lower osmotic concentration in the tracheoles to the greater concentration in the tissues. Similarly it was believed by Maloeuf (1938) that the mechanism of the flashing in fireflies results from the sudden increase in osmotic pressure in the light-producing cells, causing the absorption of the fluid from the tracheoles and giving increased access to oxygen. Wigglesworth (1938) states that osmosis did not explain the action of the fluid

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[3]This conclusion is borne out by studies of insect tracheoles under the electron microscope, in which it has been shown that spiral thickenings, or taenidia, are present in tracheoles (Richards and Anderson, 1942a, 1942b).

and postulated imbibition as the mechanism. In corroboration of this it was shown that when saline water mosquito larvae were transferred to sea water and glycerol the osmotic pressure was trebled, but the extent of the fluid in the tracheoles was unaltered (Beadle, 1939). From this it was concluded that the force must reside in the substance of the protoplasmic lining of the tracheoles. Further study is necessary to solve these problems of the mechanism of fluid movement in the tracheoles and the relation of the fluid to the metabolism of the insect.

## Summary

1. Although fluid is not normally present in the tracheal system of the larva of *Drosophila melanogaster,* its entry from the tissues may be induced by several means.

2. Absorption of the fluid may occur at any point in the tracheal system, no one part being more permeable than any other.

3. The absorption of fluid is a reversible process.

4. The entire tracheal system of the *Drosophila melanogaster* larva appears to be readily permeable to fluids and gases.

5. It is probable that there is little or no difference in the structure of the tracheae and the tracheoles of insects.

## Literature Cited

Beadle, L. C.

1939. Haemolymph in saline water mosquito larvae. *Jour. Exp. Biol.,* 16: 346.

Bridges, C. B.

1932. Apparatus and methods for *Drosophila* culture. *Amer. Nat.,* 66: 250-273.

Krogh, A.

1941. Respiratory mechanisms. Univ. of Penn. Press, Philadelphia.

Lund, E. J.

1911. On the structure, physiology, and the use of the photogenic organs, with special reference to the Lampyridae. *Jour. Exp. Zool.,* 11: 415-468.

MALOEUF, N. S. R.

1938. Quantitative studies on the respiration of the aquatic arthropods and on the permeability of their outer integument to gases. *Jour. Exp. Zool.*, 74: 323-351.

RICHARDS, A. G., JR. AND T. F. ANDERSON

1942a. Electron micrographs of insect tracheae. *Jour. New York Ent. Soc.*, 50: 147-167.

1942b. Further electron miscroscope studies on arthropod tracheae. *Ibid*, 50: 245-247.

SYKES, E. K. AND V. B. WIGGLESWORTH

1931. The first appearance of air in the tracheal system. *Quart. Jour. Micros. Sci.*, 74: 165.

WIGGLESWORTH, V. B.

1933. Anal gills of mosquito larvae. *Jour. Exp. Biol.*, 10: 1-37.

1938. The absorption of fluid from the tracheal system of mosquito larvae at hatching and moulting. *Ibid*, 15: 248-254.

1939. The principles of insect physiology. Dutton and Co., New York.